Effects of ontogeny and invasive crayfish on feeding ecology and mercury concentrations of predatory fishes

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Abstract

Lake food web structure dictates the flow of energy and contaminants to top predators, and addition of invasive species can shift these flows. We examined trophic position (TP), proportional reliance on the littoral zone ($Prop_{littoral}$), and mercury (Hg) concentrations across the life span of two predatory fishes, walleye (*Sander vitreus*) and northern pike (*Esox lucius*), in lakes with and without invasive virile crayfish (*Faxonius virilis*). The littoral was the dominant foraging zone for both species regardless of size, accounting for 59% and 80% of the diet of walleye and pike, respectively. Both species increased in TP and Hg with body size, as did crayfish. Walleye in crayfish-present lakes had lower $Prop_{littoral}$, TP and Hg concentrations compared with non-present lakes, but trophic magnification of Hg through the food web was consistent across all six lakes. These findings underscore a strong role for the littoral zone in channeling energy and contaminants to higher trophic levels, and how invasive species can occupy new habitats at low abundance while altering food web structure and contaminant bioaccumulation.

Introduction

The structure of lake food webs has important implications for energy flow and contaminant bioaccumulation (Vander Zanden et al. 1996). Both horizontal and vertical food web structure play roles in shaping energy flow from primary producers to apex predators. Fishes act as couplers of littoral and pelagic zones in lakes by deriving energy from both pathways through their mobile foraging (Schindler and Scheuerell 2002; Vander Zanden and Vadeboncoeur 2002). Meanwhile, foraging by fishes at the top of long food chains leads to high concentrations of potentially harmful compounds such as mercury (Hg) (Cabana and Rasmussen

1994) because concentrations increase roughly 5 to 7 times per trophic level (Lavoie et al. 2013). Also, differential exposure to some chemicals occurs when organisms forage in different food web compartments (Kidd et al. 2001).

Ontogeny is a key process that dictates feeding patterns in lake fishes. Many species display predictable shifts from the pelagic zone as larvae to the littoral zone as adults (King 2005; Jardine et al. 2015). This shift could affect Hg concentrations because the pelagic zone can have higher concentrations for a given trophic level (Power et al. 2002; Ethier et al. 2008). Furthermore, most predatory species exhibit increases in trophic position (TP) throughout their life span, and for some species, a switch to piscivory occurs very early in development (Mittelbach and Persson 1998; Post 2003). Progressive shifts to prey with larger sizes that occupy higher trophic levels is likely responsible for known increases in Hg concentrations with size and age in fishes (e.g. Jardine et al. 2012).

Invasive species have the potential to modulate food web structure, extend or shorten the length of food chains, and thereby affect contaminant concentrations of apex predators (Cabana and Rasmussen 1994; Vander Zanden and Rasmussen 1996). Non-native crayfish are expanding their range, affecting aquatic ecosystems in all continents except Antarctica (Phillips et al. 2009; Lodge et al. 2012). This includes slow post-glacial expansion by a widely distributed species, *Faxonius virilis* (Phillips et al. 2009). Different species of crayfish can have different mercury concentrations and energy densities even when they co-occur, suggesting predation on recently introduced species could alter concentrations in predators (Johnson et al. 2014). Work in the United States Midwest shows that lakes invaded by crayfish can have predatory fishes that feed more in the littoral zone and occupy lower trophic positions (Nilsson et al. 2012; Kreps et al. 2016). Ontogenetic shifts in diet within species could directly influence these patterns.

We determined the relative role of ontogeny and the presence of crayfish in determining feeding ecology and Hg concentrations in predatory fishes in lakes of central Saskatchewan, Canada. First, we used stable C isotopes to examine if walleye (*Sander vitreus*) and northern pike (*Esox lucius*) exhibited shifts from the pelagic zone to the littoral zone as they grew. Next, we determined patterns in TP using stable N isotopes and assessed whether any shifts were accompanied by changes in Hg concentrations. Finally, we evaluated how the presence of crayfish (*F. virilis*) affected these three biological endpoints. We conducted these analyses to help better understand the implications of changing crayfish distributions, and how individual foraging behaviour dictates contaminant concentrations.

Methods

Sampling was conducted in 2015 in six prairie lakes (Figure 1), located in South East Saskatchewan, Canada. The lakes are located in close proximity to each other, are classified as eutrophic, and share similar substrate, a mixture of mud, sand, gravel and boulder (Water Security Agency 2016) (Table 1). All are natural lakes with the exception of Theodore Lake, which is a river valley reservoir.

Crayfish have been slow to recolonize these lakes following glaciation (Phillips et al. 2009), in part because of limited hydrological connectivity to downstream waters. Populations in the region become established through a combination of natural recolonization and human assistance due to connections established by new water conveyance works. The six lakes have been actively surveyed in summer since 2007 with snorkel counts of individuals along 10 transects (1 m wide by 10 m long) in 1 – 3 m deep littoral habitats, overturning cobble and other potential refuge (Table 2). Fishing, Margo and Stoney lakes do not have documented crayfish

93 populations. In addition to snorkeling efforts, baited Gee-minnow traps were set in Stoney Lake
94 for 56 trap days in 2013, in Fishing Lake for 432 trap days in 2015, and Margo Lake for 416 trap
95 days in 2015 without catching any crayfish. This, in combination with non-detects in cobble
96 basket sets and kick and sweep surveys suggest that crayfish were absent from these lakes at the
97 time of study. Crayfish have since accessed Stoney Lake via a narrow connecting channel from
98 Whitesand Lake, with densities rising to 2 individuals/m^2 by 2018 (I. Phillips, unpublished data).

99 Crayfish are present in Whitesand, Newburn and Theodore lakes. In addition to densities
100 ranging from 1 to 44 individuals/m^2 from the snorkelling surveys (Table 2), trapping in 2013
101 yielded a catch-per-unit effort of 2.1 crayfish $trap^{-1}$ day^{-1} in Whitesand for two traps set over 20
102 days. This value is similar to that for *F. virilis* in Wisconsin and Michigan lakes (Kreps et al.
103 2016). For our other two crayfish-present lakes, kick net sweeps in the littoral zone were used as
104 the main sampling method for this study, and effort was expended only until sufficient numbers
105 were collected. Therefore we do not have minnow trap CPUE data for these lakes to compare
106 with other studies, but densities estimated from snorkeling suggest highest abundance in
107 Newburn Lake (Table 2).

108 Samples for stable isotope and Hg analysis were collected from various sites in each lake
109 in the summer of 2015 from mid-June to mid-August. Walleye, northern pike and yellow perch
110 (*Perca flavescens*) were collected by angling and using three 60-meter nylon monofilament mesh
111 gill nets with 10 m increments ranging in mesh size from 1.9 cm to 10.2 cm. Gill nets were set
112 perpendicular to shore for ~12-24 hours overnight. All individuals had their stomach contents
113 briefly inspected for large prey items, and a subsample of each species spanning a size range
114 representative of the sample was used to collect dorsal muscle tissue samples for stable isotope
115 analysis (SIA). The proportion of fish with crayfish in their stomachs in the crayfish-absent lakes

was 0 of 60 for Fishing Lake, 0 of 89 for Margo Lake and 0 of 53 for Stoney Lake. In the crayfish-present lakes, it was 4 of 79 for Whitesand, 0 of 44 for Newburn Lake and 13 of 74 for Theodore Lake. Measurements of fork length and weight were recorded for all fish species. Using minnow traps, seine netting and kick net sweeps, a size range of crayfish was sub-sampled for analysis. Kick net sweeps were conducted along the shore for three minutes at a water depth of approximately 1 m to 1.5 m to capture additional benthic macroinvertebrates. These samples were later sorted and analyzed in the laboratory with taxa keyed to the lowest possible designation. We used snails to represent the littoral zone isotope end-member. For two of the lakes we used data from a prior sampling event in 2013. To represent the pelagic zone, zooplankton were collected monthly with a Wisconsin Net with a hoop diameter of 20 cm, a length of 90 cm, and mesh size of 80 μm. Vertical tows were repeated until a sufficient sample was collected for SIA. Samples were later hand sorted in the laboratory to attain a concentrated zooplankton sample from each lake. All samples were stored frozen until analysis.

Stable Isotope Preparation and Analysis

Samples were dried at 60˚C for ~48 hours before being homogenized with a mortar and pestle. Whole organisms were dried for all samples except adult fish, where representative dorsal muscle was used. Snails were removed from their shells prior to drying. Once homogenized, subsamples were weighed into tin capsules (1.0 ± 0.1 mg). SIA was performed at the UC-Davis Stable Isotope Facility using a continuous flow isotope ratio mass spectrometer (CF-IRMS). Stable isotope ratios for nitrogen and carbon are reported in delta (δ) notation defined as parts per thousand or permil (‰) deviation from an international standard. The formula for the delta (δ) notation is as follows:

$$\delta X = [(R_{SAMPLE} / R_{STANDARD})-1]*1000$$

Where: X is the heavy isotope of the particular element being measured (either ^{15}N for nitrogen or ^{13}C for carbon); R_{SAMPLE} is the ratio of the heavy isotope to the light isotope for the particular element in the sample ($^{15}N/^{14}N$ for nitrogen or $^{13}C/^{12}C$ for carbon); $R_{STANDARD}$ is the ratio of the heavy isotope to the light isotope for an international standard (Pee Dee Belemnite limestone for $^{13}C/^{12}C$ (Craig 1957) and atmospheric nitrogen for$^{15}N/^{14}N$ (Mariotti 1983)). International standards are set at delta (δ) values of 0‰. Samples analysed in duplicate (n = 20) had an average difference of 0.3‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Calculations and Statistical Analysis

Lipid extraction for animal tissue was not conducted prior to SIA and instead a lipid correction factor (Logan et al. 2008) was applied following: $\delta^{13}C_{corr} = \delta^{13}C-[(-2.8317*LN(C/N))+2.8838]$ where: $\delta^{13}C_{corr}$ is the lipid-corrected value, $\delta^{13}C$ is the $\delta^{13}C$ value for the particular sample; -2.8317 and 2.8838 are constants; and C/N equals the elemental carbon to nitrogen ratio for the particular sample.

For each fish and crayfish, the proportion of the diet derived from the littoral zone was calculated as $Prop_{littoral} = (\delta^{13}C_{consumer} – \delta^{13}C_{pelagic})/(\delta^{13}C_{littoral} – \delta^{13}C_{pelagic})$ where $\delta^{13}C_{pelagic}$ is the $\delta^{13}C$ value of zooplankton, and $\delta^{13}C_{littoral}$ is the $\delta^{13}C$ value of snails. Trophic position was determined by accounting for different baseline $\delta^{15}N$ in the littoral and pelagic zones using: $TP = 2 + [\delta^{15}N_{consumer} – (\delta^{15}N_{littoral} \times Prop_{littoral} + \delta^{15}N_{pelagic} \times Prop_{pelagic})]/\Delta 15N$ where $\delta^{15}N_{pelagic}$ is the $\delta^{15}N$ value of zooplankton, $\delta^{15}N_{littoral}$ is the $\delta^{15}N$ value of snails, and Δ15N is the trophic enrichment factor and was estimated as 3.4‰ (Post 2002).

Mercury was analysed as total Hg dry weight using a Direct Mercury Analyser (DMA, Milestone, Inc.). Samples were weighed at 20 ± 1 mg and thermally decomposed in the DMA

before amalgamation and atomic absorption. Blanks were less than 50% of the detection limit (0.04 ng of Hg), and recoveries of two secondary certified reference materials analysed alongside samples (DORM-4 dogfish liver and IAEA-85 human hair) were 102 ± 6% (n = 24) and 95 ± 1% (n = 12).

All statistical analyses were conducted in SPSS version 25 (Chicago, IL). To assess the effects of body size on $Prop_{littoral}$, TP and total Hg concentrations, we first used ordinary least-squares regressions within lakes. To test for the effects of crayfish presence on these three variables we used a general linear model ANOVA with the random factor 'lake' nested within the fixed factor 'type' (crayfish present or absent) and length as a covariate, separately for walleye and northern pike. Mercury concentrations were log-transformed to improve normality and reduce heteroscedasticity. To calculate trophic magnification, Hg concentrations of all organisms in the food web of each of the lakes were regressed against their TPs and a regression was fit according to Log Hg = m*TP + b. The slopes of these regressions (m) were then used to calculate Trophic Magnification Factors (TMFs) using $TMF = 10^m$ (Fisk et al. 2001) where TMF represents the average increase in Hg concentration for each TL (Lavoie et al. 2013). Slopes and intercepts were compared among lakes using an Analysis of Covariance with lake as the factor, log Hg as the response variable, and TP as the covariate.

Results

Zooplankton and snails appropriately bracketed the $\delta^{13}C$ values for the fishes (Figure 2). Across all lakes, mean zooplankton $\delta^{13}C$ was -31.4 ± 2.8‰ S.D. (n = 77) while snails had $\delta^{13}C$ = -27.6 ± 2.0‰ S.D. (n = 131). Within lakes, the average difference in $\delta^{13}C$ between the pelagic and littoral was 4.1 ± 1.1‰ S.D. The $\delta^{15}N$ value of the two baseline organisms were variable

184 across lakes but had similar mean values overall: zooplankton $\delta^{15}N = 8.8 \pm 1.9‰$ S.D. (n = 77),
185 snail $\delta^{15}N = 8.2 \pm 1.6‰$ S.D. (n = 131).

186 Walleye and pike showed strong dependence on the littoral zone in five of the six lakes.
187 Only Theodore Lake had $Prop_{littoral} < 0.5$ for both species, and $Prop_{littoral}$ averaged 0.59 and 0.80
188 overall in walleye and pike, respectively (Table 3, Figure 3). There was limited evidence for
189 ontogenetic shifts from the pelagic to the littoral, with only walleye in two lakes (Fishing Lake
190 and Margo Lake) having significant relationships between $Prop_{littoral}$ and body size (Table 4).
191 Crayfish, when present, had highly variable $\delta^{13}C$ and $Prop_{littoral}$ across lakes, ranging from <0.0
192 to >100.0, suggesting error in the estimation of end-member values, feeding on other sources
193 (e.g. terrestrial inputs) or trophic enrichment outside of typical ranges. Like the two fish species,
194 crayfish in Theodore Lake were strongly dependent on the pelagic zone. In walleye, there were
195 significant differences in crayfish-present and crayfish-absent lakes, with crayfish-present lakes
196 having lower $Prop_{littoral}$ ($F_{1,112} = 141.762$, $p < 0.001$), driven largely by low values in Theodore
197 Lake. For walleye, length was a significant predictor of $Prop_{littoral}$ ($F_{1,112} = 7.815$, $p = 0.006$),
198 with larger individuals having higher $Prop_{littoral}$, but this was not the case for pike ($F_{1,72} = 0.312$,
199 $p = 0.578$). Lake type also had no effect on $Prop_{littoral}$ for pike ($F_{1,72} = 0.075$, $p = 0.784$).

200 Both fish species occupied elevated TPs indicative of tertiary consumers, with walleye
201 (3.94 ± 0.35) having slightly higher mean values than pike (3.77 ± 0.40) and both species
202 approximately 0.5 to 1.0 TPs above yellow perch that had mean TP = 3.21 ± 0.23. Walleye
203 increased almost two TPs over the size range that we captured, but the pattern for pike was less
204 clear (Table 4, Figure 4). Overall TPs for walleye, after accounting for the significant effect of
205 body size as a co-variate ($F_{1,111} = 101.357$, $p < 0.001$), were slightly lower in crayfish-present
206 lakes (marginal mean TP = 3.88 ± 0.03 SE) compared with crayfish-absent lakes (TP = $4.00 \pm$

0.02 SE) ($F_{1,111} = 10.916$, $p < 0.001$), while differences for pike were not significant ($F_{1,72} = 0.157$, $p = 0.693$). Crayfish were uncommon in the stomachs of both species, appearing in only two of the 105 walleye and only one of the 46 northern pike examined in crayfish-present lakes, respectively. Instead, crayfish appeared more often in the diets of yellow perch, with 15 of 45 individuals having crayfish in their stomachs.

Mercury concentrations were high in both species, exceeding the Health Canada guideline of 0.5 µg/g wet weight in 41% (140 of 343) of cases (Figure 5). Mean concentrations for walleye were above the guideline in four of the six lakes, but none of the lakes had pike with mean concentrations above the guideline (Table 3). Mercury concentrations increased consistently with body size for both species (Table 5), and also for crayfish (Figure 6). For the latter species, this was likely due to increased TP in larger individuals (Figure 6). Both length ($F_{1,242} = 234.481$, $p < 0.001$) and lake type ($F_{1,242} = 31.238$, $p < 0.001$) had significant effects on Hg concentrations in walleye, with lower values in the crayfish-present lakes (marginal mean = 2.1 µg/g dry weight) compared with crayfish-absent lakes (1.6 µg/g dry weight). While length also significantly affected Hg concentrations in pike ($F_{1,80} = 99.454$, $p < 0.001$), there was no crayfish effect ($F_{1,80} = 0.772$, $p = 0.382$).

Trophic magnification of Hg through the food web was strong and consistent across all lakes (Figure 7). Trophic magnification factors ranged from 3.7 in Stoney Lake to 5.0 in Margo Lake (Table 6). The interaction term (lake x TP) in the model was not significant ($F = 1.805$, $p = 0.111$), suggesting parallel slopes and equivalent TMFs across lakes regardless of the presence of crayfish. After removing the interaction term, TP was significant ($F = 1652.86$, $p < 0.001$) and there was also a significant lake effect ($F = 5.977$, $p < 0.001$). Post-hoc comparisons indicated

229 that Fishing Lake had a significantly higher marginal mean than the other five lakes, which did
230 not differ from each other.
231
232 **Discussion**
233 Crayfish were associated with unexpected effects on the habitat foraging of top predators
234 in our study lakes. Past work revealed a greater contribution of the littoral zone (benthos) to the
235 diet of piscivorous fishes when invasive crayfish were in great abundance (Nilsson et al. 2012;
236 Kreps et al. 2016). In our case, crayfish presence was associated with more pelagic foraging
237 rather than littoral foraging, contrary to this earlier work. Much of this was owing to Theodore
238 Lake, where walleye and pike both fed more in the pelagic zone and at lower trophic levels
239 compared with other lakes, akin to lake trout shifts following bass invasion (Vander Zanden et
240 al. 1999). Crayfish in Theodore Lake also had the lowest trophic level and lowest littoral
241 contribution, suggesting that the food web in this lake is based more on the pelagic zone. This
242 lake is a long, narrow river valley reservoir formed by the construction of Theodore Dam.
243 Brinkmann and Rasmussen (2010) showed that pike aligned more closely with zooplankton in a
244 narrow Prairie reservoir, and in reservoirs such as this with large shoreline water level
245 fluctuations, benthic production can be compromised leading to greater use of the pelagic zone
246 by fishes (Black et al. 2003). This indicates that lake shape and hydrological regime may be
247 more important than community membership in driving fish foraging patterns (Dolson et al.
248 2009), and warrants further investigation.
249 Crayfish forage extensively on benthic macroinvertebrates and they can appear in the diet
250 of fishes, suggesting they could add a trophic level to the food chain (Phillips et al. 2009; Nilsson
251 et al. 2012). Yet Kreps et al. (2016) reported a lower TP for walleye when invasive rusty

252 crayfish were present in large numbers, consistent with our findings for *F. virilis*. In our case,
253 the two top predators did not feed on crayfish, instead consuming mostly small fish, *Gammarus*
254 sp., or having empty stomachs. Only yellow perch preyed heavily on small crayfish, particularly
255 in Theodore Lake, but this did not lengthen the food chain in crayfish-present lakes since yellow
256 perch were also generally not consumed by walleye and pike. Larger crayfish escape predation
257 (Dorn and Mittelbach 1999; Hein et al. 2006) and therefore can act as a trophic "dead-end" in the
258 food web (Cremona et al. 2008) rather than contributing to biomass production at higher trophic
259 levels and lengthening food chains.

260 Crayfish occupied TPs that were approximately one level above herbivores, and TP
261 increased with body size in all three lakes where they were present. This is consistent with
262 earlier studies that concluded crayfish were predators (Whitledge and Rabeni 1997; Roth et al.
263 2006), and could also indicate that predation on small fish occurs in largest individuals that had
264 TP > 3.0. It is unknown if crayfish in these systems deplete benthic macroinvertebrate
265 populations upon invasion, as has been demonstrated elsewhere (Nilsson et al. 2012), but their
266 relatively low abundance suggests this is unlikely. Crayfish Hg concentrations (mean = 0.32 µg/g
267 dry weight for all lakes) were within the reported range of 0.1 to 1.4 µg/g dry weight (Mueller
268 and Serdar 2002; Kouba et al. 2010) and significant, positive relationships between size/age and
269 Hg concentrations, as we observed here, are common in crayfish populations (Allard and Stokes
270 1989).

271 We found little evidence for ontogenetic habitat shifts in our two study fish species. The
272 littoral zone accounted for ~60% of the diet of both species across all body sizes, consistent with
273 earlier estimates for lake fish populations (Vander Zanden and Vadeboncoeur 2002) and further
274 pointing to the littoral zone as deserving of equal attention as the pelagic zone in governing lake-

wide productivity. The littoral contribution to diet was higher than expected for both species. Vander Zanden and Vadeboncoeur (2002) previously reported a limited contribution of zoobenthos to the diets of these species based on gut contents (14%), where isotope studies were limited. However, as piscivores, walleye and pike were believed to consume benthic prey indirectly by eating fishes from the littoral zone, accounting for ~45% of their diet (Vander Zanden and Vadeboncoeur 2002). Our data suggest that this is the case. Elsewhere, both species were strongly aligned isotopically with benthic prey in boreal shield lakes (Bertolo et al. 2005) and % littoral was as high as 96% and never lower than 68% for walleye in four Michigan lakes (Herbst et al. 2016). These findings highlight the importance of the littoral zone as a source of energy and contaminants to higher trophic levels, and how contamination of the nearshore environment from industrial activity can lead to high concentrations of toxic chemicals in the tissues of top predators (Eagles-Smith et al. 2008).

Both species had lower mean TPs compared to literature summaries (Vander Zanden et al. 1997; Paradis et al. 2008; Depew et al. 2013a), which is supported by gut content observations for these lakes and dietary flexibility to include non-fish prey (Beaudoin et al. 1999; Venturelli and Tonn 2005; Paradis et al. 2008). Yet despite these relatively low TPs, Hg concentrations were often above guidelines and greater than median concentrations for the two species summarized elsewhere (Depew et al. 2013a). This could be related to slow growth in these species near the northern edge of their distribution (Lavigne et al. 2010). Both walleye and northern pike increased their TP as they grew, leading to higher Hg concentrations in larger, older fish. The strength of these relationships varied across lakes, suggesting a decoupling of body size, TP and Hg concentrations at the individual level in some lakes. Increases in TP with body size were consistent with expectations that increasing gape size allows consumption of

larger, higher trophic level prey as fishes attain larger body sizes (Mittelbach and Persson 1998). Others have argued that ^{15}N could accumulate with age in long-lived fishes independent of dietary switches to higher trophic levels (Overman and Parrish 2001), but stomach data showed that the largest walleye in our sample set tended to have fish in their stomachs, further supporting their position as apex predators in these lakes.

There are three possible processes that lead to differential Hg concentrations in top predators, including stronger trophic magnification through the food web, longer food chains and higher baseline Hg concentrations (Kidd et al. 2012). Trophic magnification factors differed little among lakes, and were very near the global average of 4.7 for total Hg (Lavoie et al. 2013). Given the similarities in species composition and their geographic proximity to one another, it is not surprising that TMFs fell in such a narrow range. Food chain length was also similar at all lakes, with large walleye occupying the highest TP, though Theodore Lake walleye and pike had lowest mean TPs and consequently the lowest total Hg concentrations. A higher baseline concentration may be responsible for the higher marginal mean observed in Fishing Lake, where significant flooding had occurred in the two years prior to sampling (Water Security Agency 2016). This lake also had the highest concentrations in zooplankton and snails at the base of the food web. The many individual walleye and pike above the Hg consumption guideline highlight how prairie lakes are not immune to Hg contamination despite many suggesting that Western North America has lower atmospheric Hg deposition (Prestbo and Gay 2009) and hence lower risk to fish-eating consumers (Depew et al. 2013b). Concentrations in the two species were higher in all six of our study lakes compared with historical provincial records for these lakes (Depew et al. 2013a). This could owe, in part to recent high water levels in the region (Water Security Agency 2016) as flooding of soils and associated organic matter is known to elevate

baseline Hg concentrations in shallow flooded areas (Hall et al. 2009; Watras et al. in press). While we have shown that crayfish invasion appears to have modest effects on food web structure and Hg bioaccumulation, the productive littoral zones of these lakes are clearly transferring Hg to higher trophic levels.

Our work shows effects of *F. virilis* on food web properties, but other species may have stronger effects. For example, rusty crayfish (*F. rusticus*) likely modify habitats (Wilson et al. 2004; Phillips et al. 2009) more strongly than *F. virilis* in part because they achieve higher relative abundance (Kreps et al. 2016). Because there are little differences among species in their overall effects (Twardochleb et al. 2013), abundance becomes a key element of an invasive species' potential to modify ecosystems (Hansen et al. 2013). In our lakes, CPUE of crayfish was well below that measured for *F. rusticus* in Wisconsin lakes (Nilsson et al. 2012; Kreps et al. 2016), where catch rates were typically 20-40 individuals per trap per day and as high as 70 individuals per trap per day. As such, provided that invasive crayfish remain at low relative abundance, their food web effects are likely to be modest. We recommend additional paired studies that include measurements of contaminants such as mercury, with more crayfish species such as *F. rusticus* that achieve higher abundances (Wilson et al. 2004; Olden et al. 2006). Such studies will reveal whether we should anticipate additional changes to food webs and contaminant risks as animal distributions continue to change across the landscape.

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507 **Table 1.** Characteristics of the six lakes studied

Lake	Lat	Long	Crayfish	Area (ha)	Mean Depth (m) (max)	TP (mg/L)	TN (mg/L)
Fishing Lake	51.833	-103.533	Absent	3967	25 (NA)	0.060	1.3
Margo Lake	51.816	-103.363	Absent	250	NA (5)	0.090	1.5
Stoney Lake	51.788	-103.364	Absent	286	5 (8)	0.070	1.7
Whitesand Lake	51.764	-103.345	Present	495	3 (11)	0.050	1.6
Newburn Lake	51.693	-103.177	Present	156	NA (6)	0.050	1.6
Theodore Lake	51.453	-102.845	Present	304	NA (NA)	NA	NA

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527 **Table 2.** Estimated crayfish densities (individuals/m^2) from snorkel surveys (10 transects per lake) in the six lakes. Empty cells
528 indicate that the lake was not surveyed in that year.

	Year								
Lake	2007	2008	2009	2010	2011	2012	2013	2014	2015
Fishing Lake	0	0	0		0	0	0		0
Margo Lake	0	0	0		0	0	0	0	0
Stoney Lake	0	0	0		0	0	0	0	0
Whitesand Lake		3.3 ± 2.5	4.3 ± 2.1		2.2 ± 2.0	3.2 ± 2.0	2.2 ± 1.8	2.2 ± 1.7	2.0 ± 2.2
Newburn Lake			26.0 ± 32.7		43.5 ± 25.8	21.6 ± 22.6	20.2 ± 17.7		25.4 ± 21.5
Theodore Lake			16.4 ± 19.0						

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533 **Table 3.** Mean (± S.D.) body size, trophic position, proportion littoral dependence ($Prop_{littoral}$)
534 and total mercury concentrations of walleye and northern pike in six lakes in the Northern Great
535 Plains. Assuming 75% moisture, the mercury guideline for human consumption is 2.0 ug/g dry
536 weight.

Walleye

Lake	Fork length (cm)	TP	$Prop_{littoral}$	[Hg] (ug/g dry wgt)
Fishing Lake	43.0 ± 3.6	4.07 ± 0.15	0.78 ± 0.09	2.83 ± 1.05
Margo Lake	41.0 ± 5.0	3.81 ± 0.29	0.72 ± 0.14	1.92 ± 0.90
Stoney Lake	40.7 ± 8.0	4.07 ± 0.37	0.53 ± 0.10	2.04 ± 0.58
Whitesand Lake	44.7 ± 4.7	4.09 ± 0.20	0.72 ± 0.06	2.08 ± 0.68
Newburn Lake	46.3 ± 9.5	3.82 ± 0.24	0.58 ± 0.12	2.24 ± 0.89
Theodore Lake	42.6 ± 11.6	3.73 ± 0.36	0.04 ± 0.08	1.80 ± 1.19

Pike

Lake	Fork length (cm)	TP	$Prop_{littoral}$	[Hg] (ug/g dry wgt)
Fishing Lake	51.2 ± 14.3	3.50 ± 0.26	0.75 ± 0.08	1.56 ± 0.98
Margo Lake	57.1 ± 5.6	3.58 ± 0.24	0.69 ± 0.12	1.38 ± 0.44
Stoney Lake	52.7 ± 3.4	4.12 ± 0.27	0.80 ± 0.17	1.99 ± 0.62
Whitesand Lake	51.6 ± 8.7	3.84 ± 0.41	0.79 ± 0.10	1.69 ± 0.66
Newburn Lake	52.6 ± 6.3	4.02 ± 0.31	0.95 ± 0.21	1.97 ± 0.54
Theodore Lake	39.9 ± 10.0	3.18 ± 0.34	0.45 ± 0.16	0.84 ± 0.30

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547 **Table 4.** Relationship between trophic position and fork length for walleye and pike in the six
548 study lakes, with regression equations for significant regressions.

Walleye

Lake	Equation	r2	p	n
Fishing Lake	Trophic position = 0.030*fork length+2.72	0.44	0.001	20
Margo Lake	Trophic position = 0.025*fork length+2.78	0.34	0.001	27
Stoney Lake	Trophic position = 0.031*fork length+2.90	0.75	<0.001	19
Whitesand Lake	Trophic position = 0.024*fork length+3.03	0.38	0.001	26
Newburn Lake	Trophic position = 0.015*fork length+3.16	0.46	0.045	9
Theodore Lake	Trophic position = 0.032*fork length+2.52	0.64	<0.001	18

Pike

Lake	Equation	r2	p	n
Fishing Lake	Trophic position = 0.011*fork length+2.95	0.48	0.002	17
Margo Lake		0.02	0.723	10
Stoney Lake	Trophic position = -0.066*fork length+7.59	0.60	0.041	7
Whitesand Lake	Trophic position = 0.037*fork length+1.92	0.62	<0.001	21
Newburn Lake		0.12	0.186	20
Theodore Lake		0.22	0.532	4

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562 **Table 5.** Relationship between log total Hg concentration and fork length for walleye and pike in
563 the six study lakes, with regression equations for significant regressions

Walleye

Lake	Equation	r2	p	n
Fishing Lake	Log Hg = 0.036*fork length-1.129	0.64	<0.001	31
Margo Lake	Log Hg = 0.036*fork length-1.244	0.53	<0.001	77
Stoney Lake	Log Hg = 0.011*fork length-0.142	0.52	<0.001	45
Whitesand Lake	Log Hg = 0.016*fork length-0.421	0.35	<0.001	51
Newburn Lake	Log Hg = 0.017*fork length-0.465	0.97	<0.001	9
Theodore Lake	Log Hg = 0.019*fork length-0.648	0.85	<0.001	35

Pike

Lake	Equation	r2	p	n
Fishing Lake	Log Hg = 0.014*fork length-0.567	0.68	<0.001	25
Margo Lake		0.32	0.086	10
Stoney Lake	Log Hg = 0.043*fork length-1.994	0.60	0.041	7
Whitesand Lake	Log Hg = 0.019*fork length-0.768	0.62	<0.001	21
Newburn Lake	Log Hg = 0.009*fork length-0.172	0.22	0.036	20
Theodore Lake		0.75	0.135	4

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577 **Table 6** Best-fit equations for Log total Hg versus Trophic Position (TP) for six Northern Great
578 Plains lakes.

Lake	Equation	r2	p	n
Fishing Lake	Log Hg = 0.700*TP-2.347	0.94	<0.001	57
Margo Lake	Log Hg = 0.703*TP-2.531	0.82	<0.001	56
Stoney Lake	Log Hg = 0.567*TP-2.052	0.85	<0.001	36
Whitesand Lake	Log Hg = 0.659*TP-2.405	0.85	<0.001	65
Newburn Lake	Log Hg = 0.662*TP-2.429	0.82	<0.001	71
Theodore Lake	Log Hg = 0.696*TP-2.546	0.78	<0.001	53

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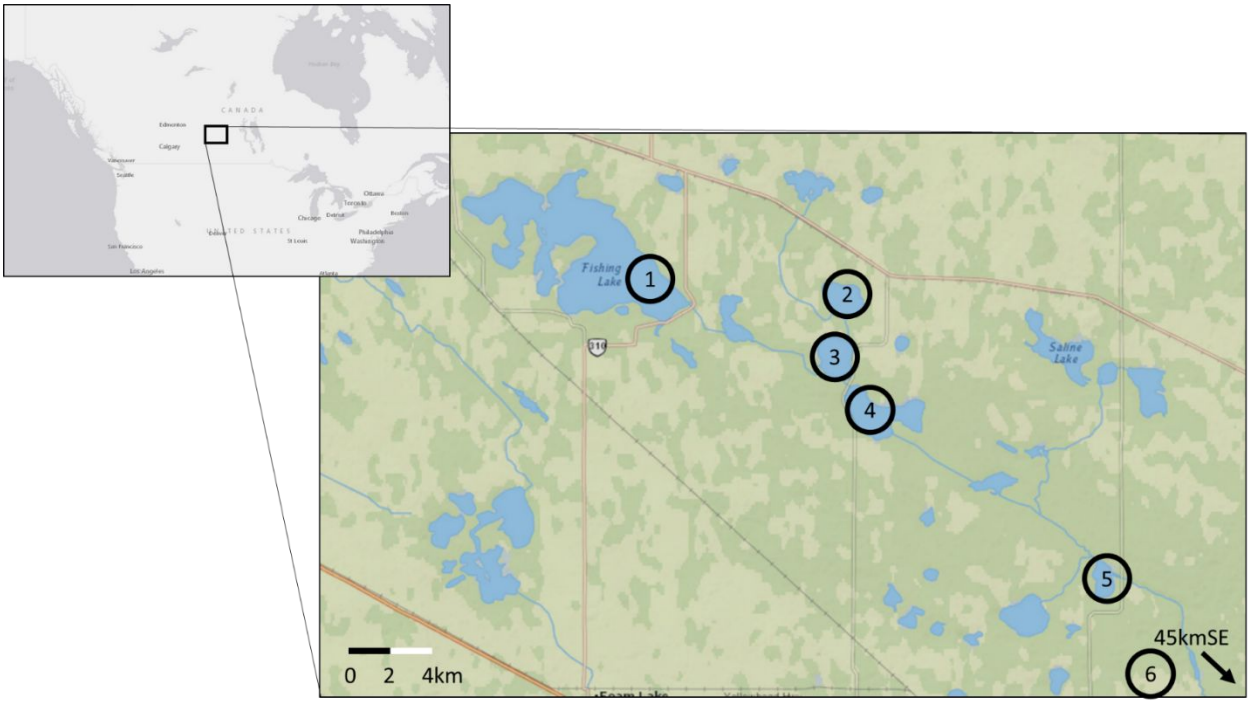
598 **Figure 1.** Location of lakes with crayfish (1 – Fishing Lake, 2 – Margo Lake, 3 – Stoney Lake)
599 and without crayfish (4 – Whitesand Lake, 5 – Newburn Lake, 6 – Theodore Lake, not shown) in
600 the Northern Great Plains.



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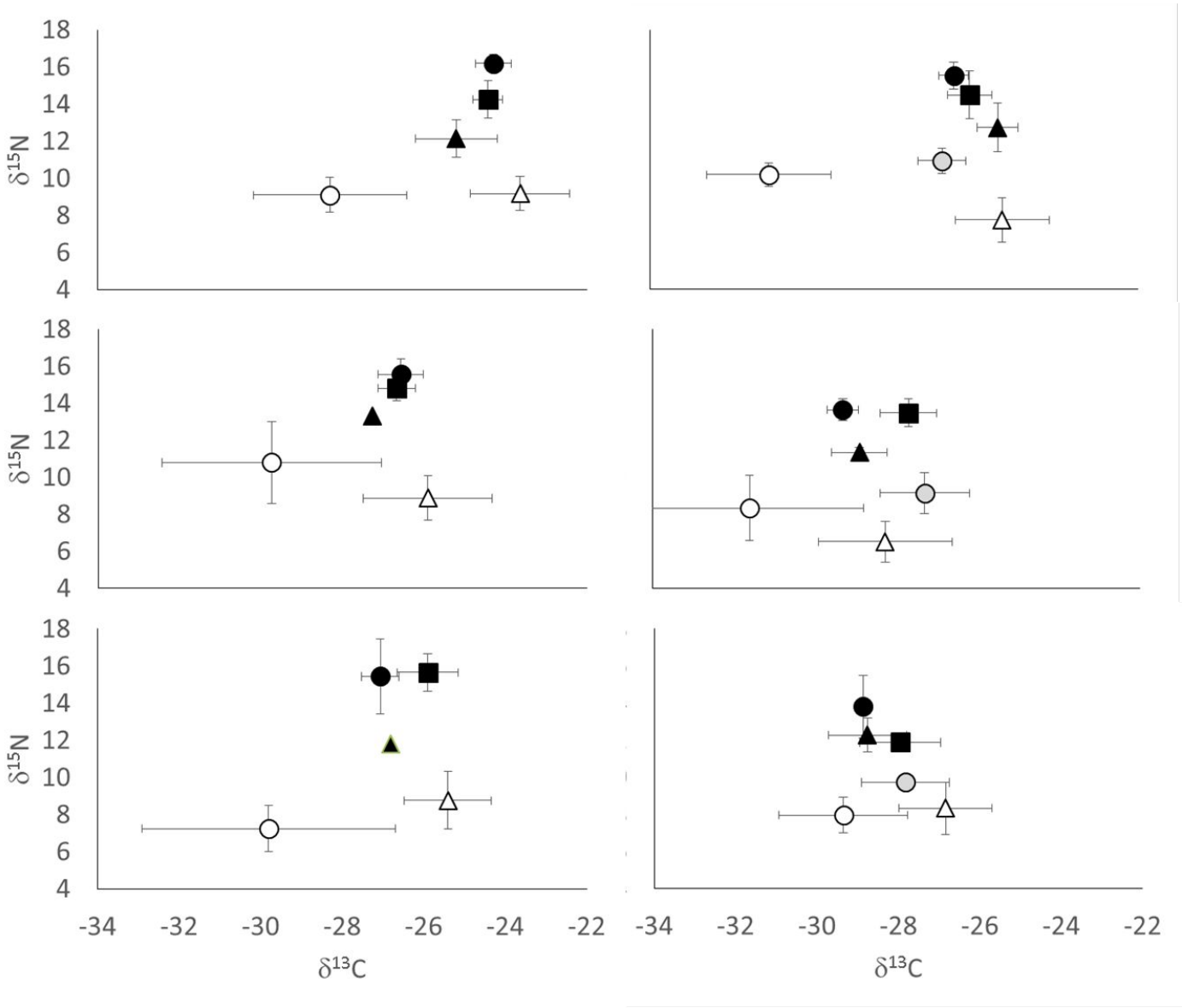
613 **Figure 2.** Scatterplot of $\delta^{13}C$ and $\delta^{15}N$ values for the food webs of the six lakes. Solid symbols
614 are fish (circles = walleye, squares = pike, triangles = yellow perch), open symbols are baseline
615 organisms (circles = zooplankton, triangles = snails) and shaded circles are crayfish.



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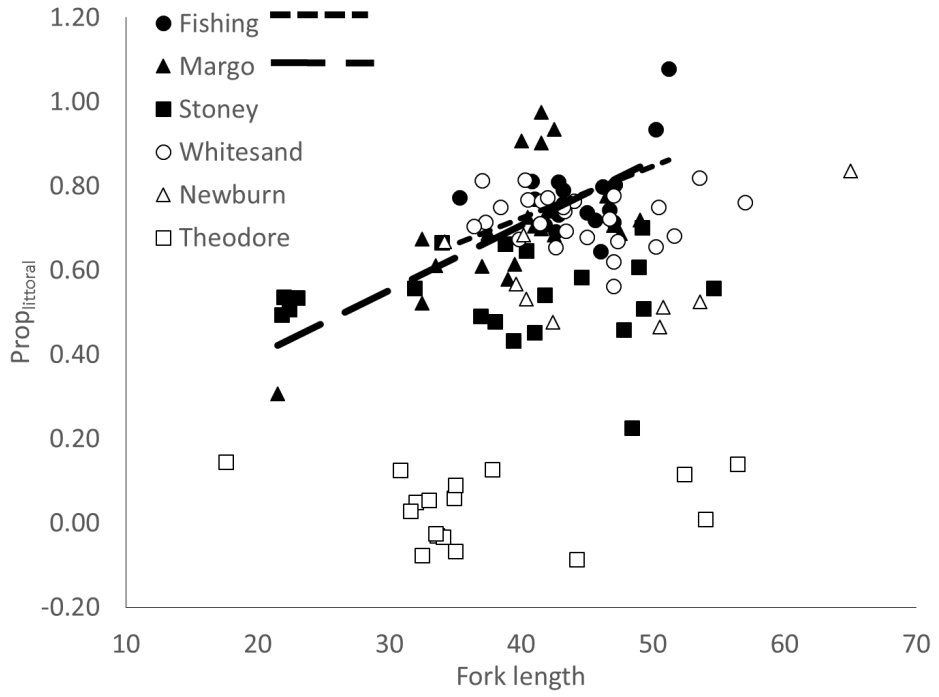
621 **Figure 3.** Proportional littoral dependence ($Prop_{littoral}$) vs. body size for walleye (A) and northern
622 pike (B) in northern Great Plains lakes with (open symbols) and without (solid symbols)
623 crayfish.



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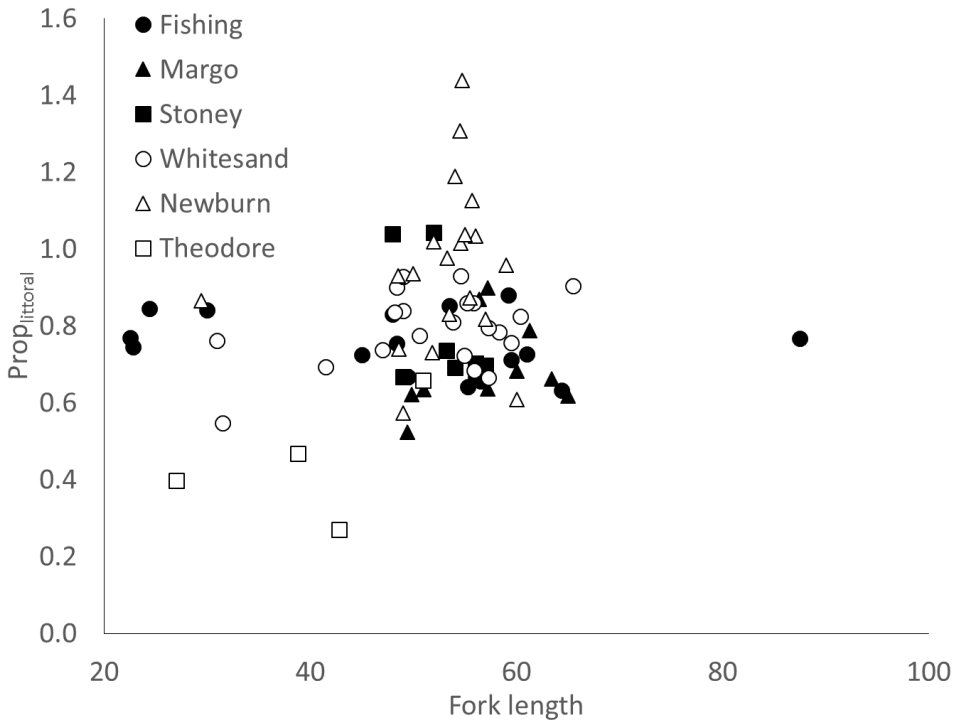

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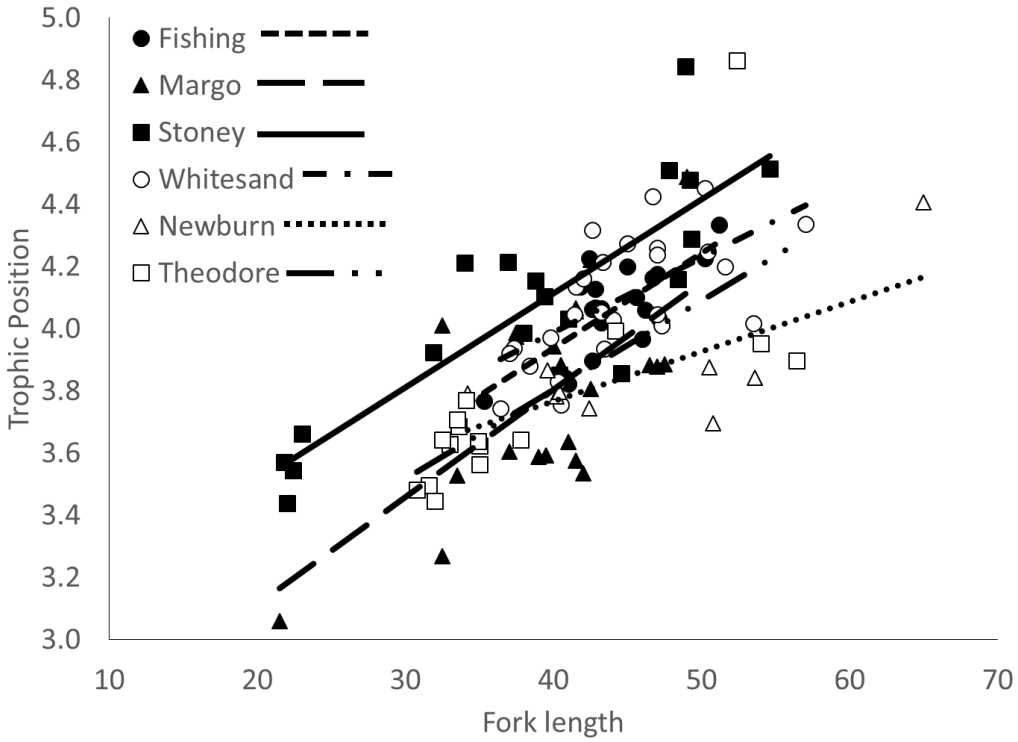
626 **Figure 4.** Trophic position vs. body size for walleye (A) and northern pike (B) in northern Great
627 Plains lakes with (open symbols) and without (solid symbols) crayfish.



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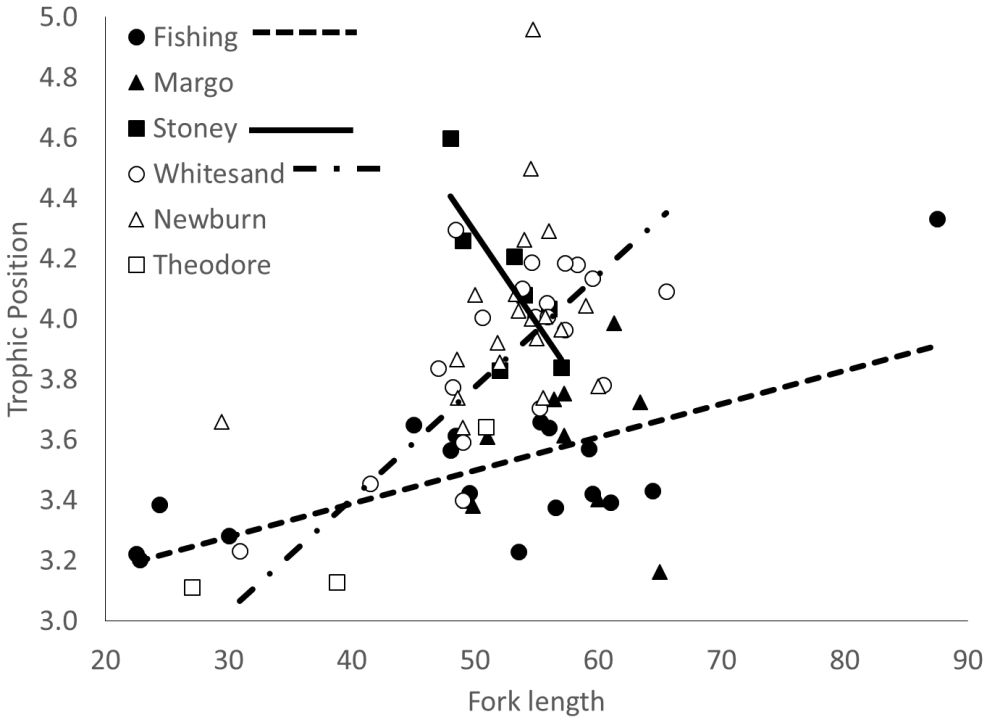

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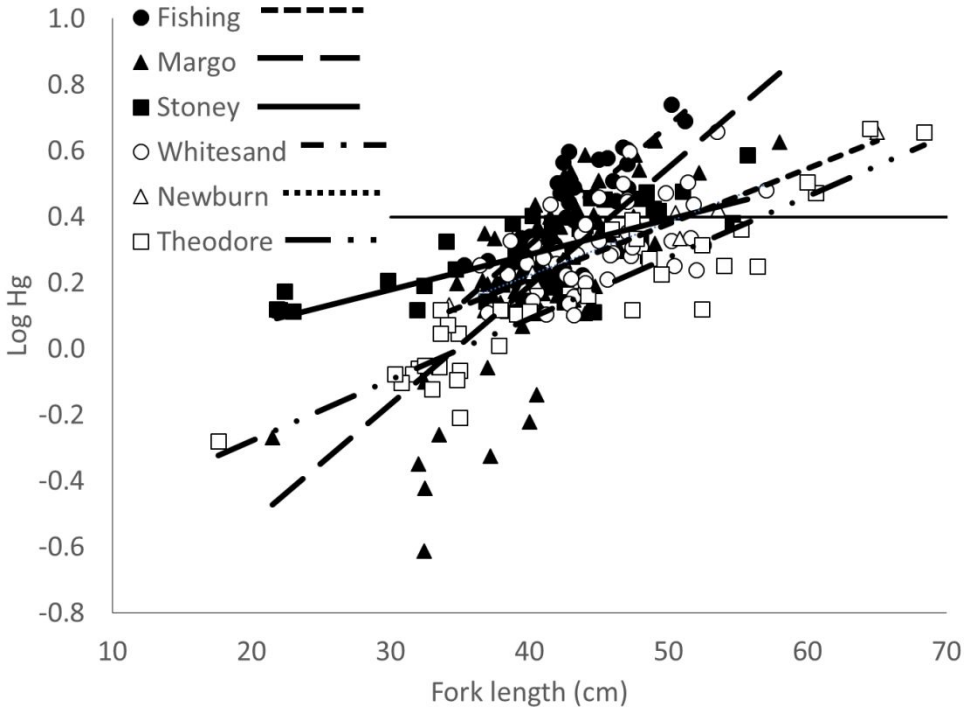
630 **Figure 5.** Mercury vs body size for walleye (A) and northern pike (B) in northern Great Plains
631 lakes with (open symbols) and without (solid symbols) crayfish.



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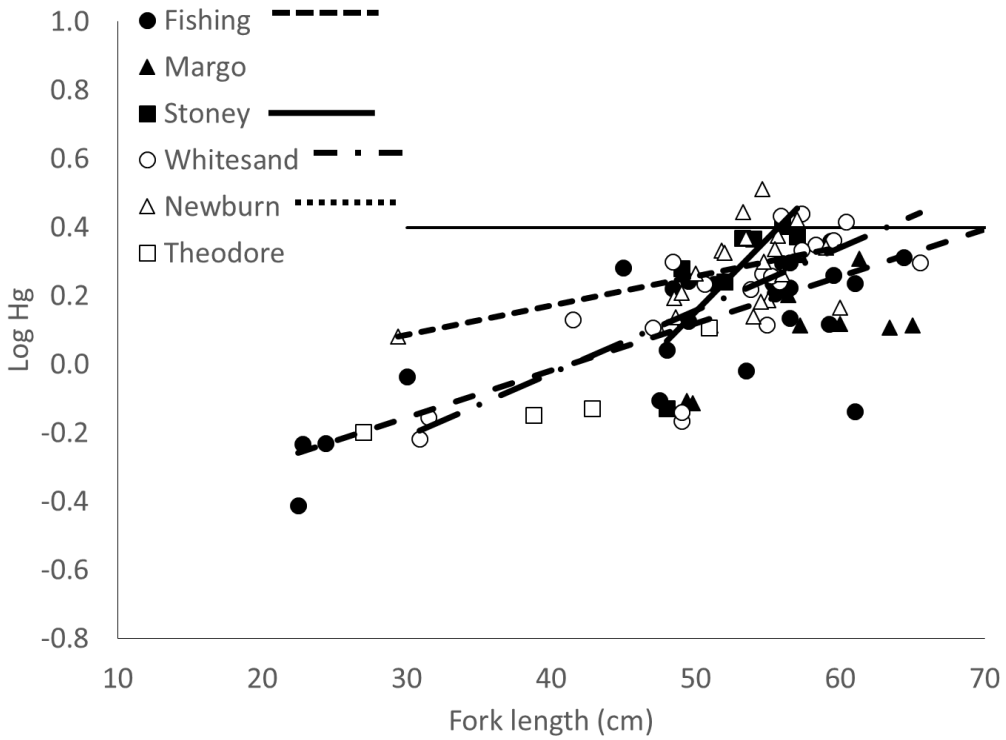

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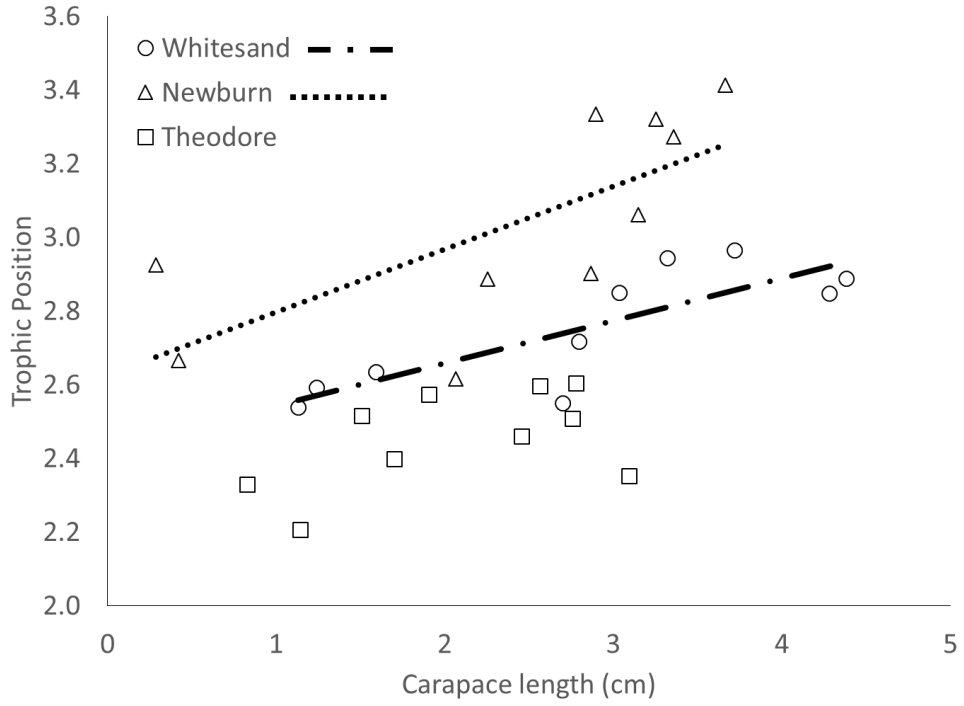
634 **Figure 6** Trophic position and log total Hg concentrations in crayfish versus body size in three
635 northern Great Plains lakes. Significant regressions are indicated with best fit lines.



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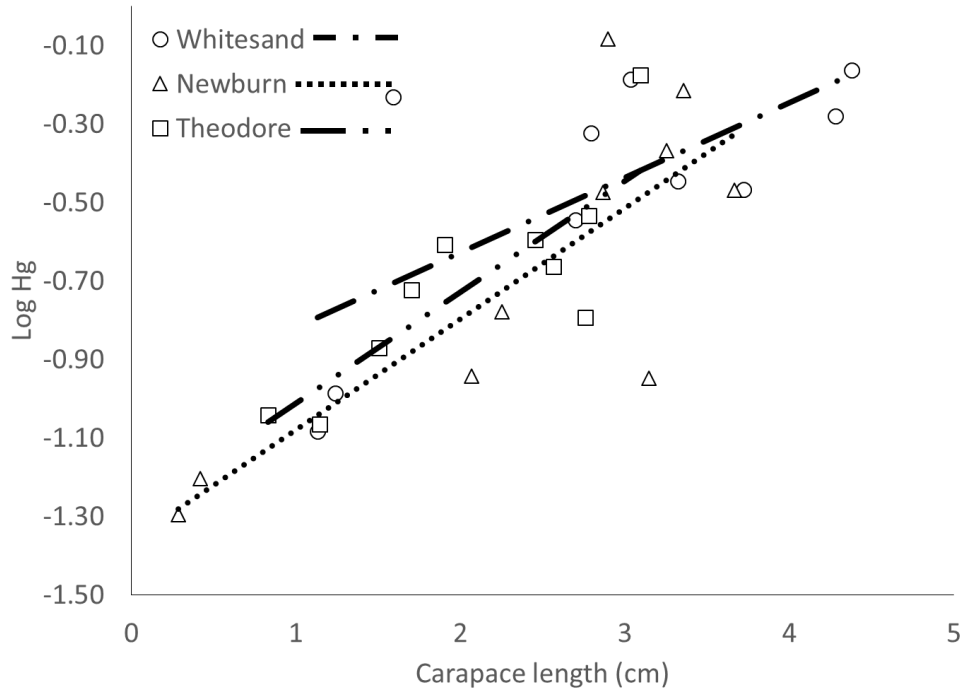

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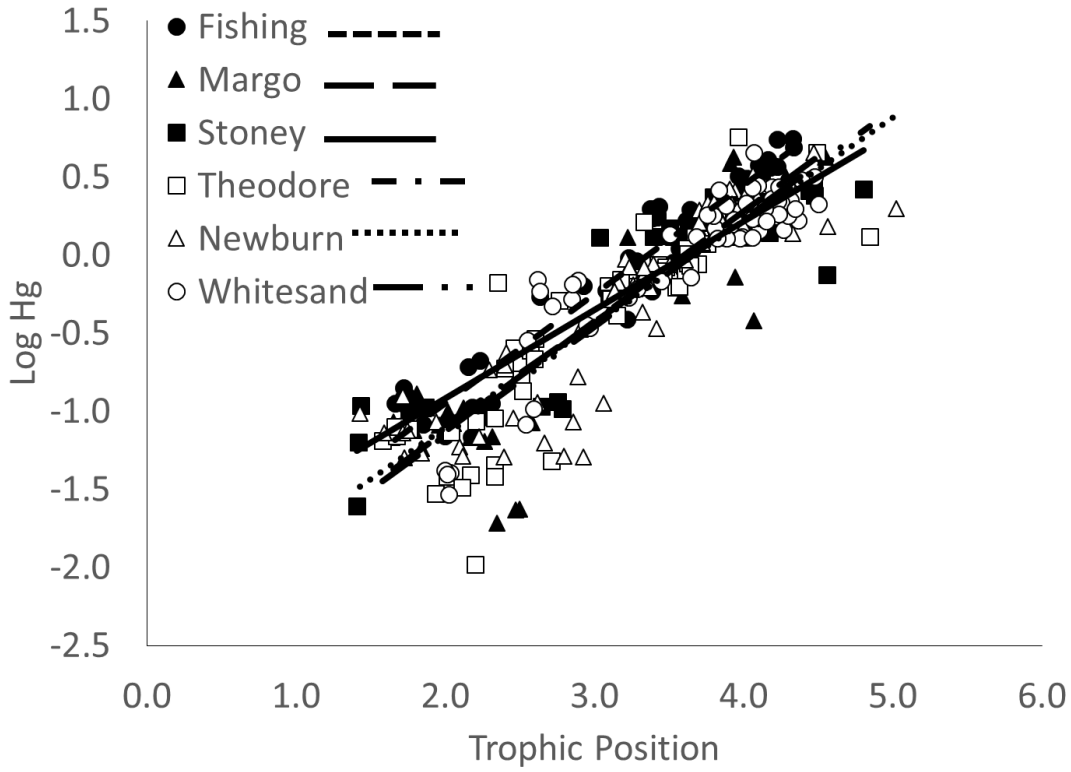
639 **Figure 7** Log total Hg concentrations versus trophic position in northern Great Plains lakes with
640 (open symbols) and without (solid symbols) crayfish.



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